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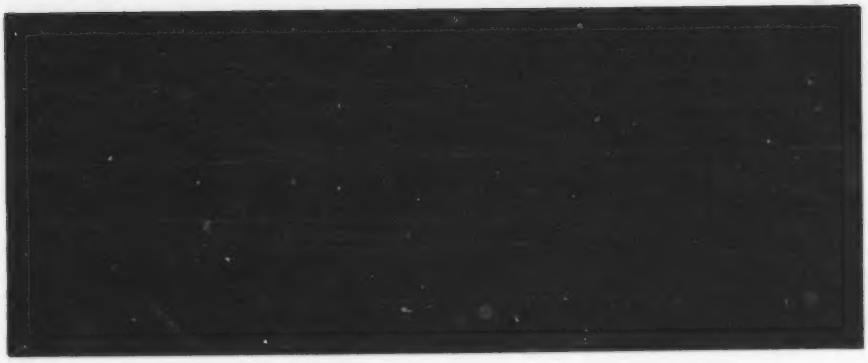

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Field Testing of a New Strain of Leafcutting Bees

Final Report - July 7/89

G.H. Rank

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Field Testing of a New Strain of Leafcutting Bees

G.H. Rank

ABSTRACT

Two varieties of the leafcutting bee Megachile rotundata were evaluated over a three-year period (1986-88) in the Saskatoon area. Both varieties of bees (Canadian commercial and French univoltine) gave similar population increases and were effective pollinators of alfalfa. The French univoltine strain showed no second-generation cells under field conditions. The French univoltine trait was also apparent from fall, winter, and spring incubation experiments. August harvested bees were chilled at 10°C for varying times, and incubated at 30°C until emergence. All three incubations resulted in a highly significant delayed emergence of the French univoltine variety compared to the Canadian commercial variety. We conclude that the French univoltine trait is maintained under Saskatchewan conditions and could be a useful management tool to assist in the control of second generation.

Field Evaluation of a French Univoltine Strain of Leafcutting Bees

G.H. Rank

A strain of leafcutting bees was imported from France in 1983 and field released near Saskatoon under quarantine conditions. Bees were raised in isolation from commercial strains and thus the genetic purity of the French strain was maintained. By 1985 successive yearly increases of the French population resulted in sufficient bees to evaluate in comparative field tests with normal commercial strains. Our main objective was to determine whether the univoltine trait of the French strain was maintained under our environmental conditions. Canadian commercial strains are bivoltine and thus can exhibit a high percentage of second generation bees in southerly latitudes ($44^{o}N$). In contrast, the French univoltine strain has been reported to produce only one generation in these latitudes. Since our climatic conditions do not result in a high percentage of second generation we evaluated the related trait of diapause intensity. Diapause intensity is defined as the time required under given conditions before the end of diapause is observed. Diapause is an arrested state of development which in the alfalfa leafcutting bee results in dormancy during the prepupal stage. Diapause is gradually broken during winter cold storage or under field conditions during cool night temperatures. A bivoltine strain would be expected to break diapause more readily than a univoltine strain. Precocious breakage of diapause under field conditions results in a field emergent second generation. The univoltine strain was expected to remain dormant for a longer period than the bivoltine strain and thus exhibit a higher diapause intensity.

Bivoltine and univoltine strains of bees were reared in similar field locations for 3 years. Cross contamination of strains was prevented by plot isolation of at least 2 km. For each strain the average time to emergence was determined for each of three seasonal periods: (i) fall incubation - initiated in September immediately after fall harvest of cells; (ii) winter incubation - initiated in December; and (iii) spring incubation - iniated in April. Population sizes varied from 200 to 2000 cells depending upon the year and time period being being evaluated. Thus all data were analyzed by an analysis by an analysis of variance with unequal population size.

A summary of three years of data is given in Table 1.

Table 1. Average emergence times during the fall, winter and spring incubation of univoltine and commercial bees in 1985, 1986 and 1987.

		Ave. Days to Emergence			
Incubation		Univoltine		Commercial	
YEAR	Period	Males	females	males	females
1986	Fall	42.8	47.9	35.5	36.3
1987	"	45.1	54.0	35.3	40.5
1985	Winter	43.0	50.6	30.0	33.9
1986	"	45.4	52.3	36.3	38.6
1987	"	42.5	49.3	34.7	38.9
1985	Spring	23.8	26.9	21.3	22.9
1986	"	25.0	29.1	22.6	24.9
1987	"	28.9	33.8	24.0	27.5

Treatment means within sexes of the two different strains were compared by a one-way analysis of variance. For each of the eight incubation periods the probability of a chance difference in mean emergence date between males and females of the two different strains was <0.0001.

For all of the inter strain comparisons at the 8 incubation periods there was a significantly higher diapause intensity for the French strain. For example, in the fall 1986 incubation the average emergence time for univoltine males was 42.8 days compared to 35.5 days for commercial males. Similarly the average emergence time of French females was 47.9 days compared to 36.3 days for commercial females.

We conclude that the trait of increased diapause intensity associated with the French univoltine trait is heritable under Saskatchewan environmental conditions.

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18(a) ABSTRACT

Two varieties of the leafcutting bee Megachile rotundata were evaluated over a three-year period (1986-88) in the Saskatoon area. Both varieties of bees (Canadian commercial and French univoltine) gave similar population increases and were effective pollinators of alfalfa. The French univoltine strain showed no second-generation cells under field conditions. The French univoltine trait was also apparent from fall, winter, and spring incubation experiments. August harvested bees were chilled at 10°C for varying times, and incubated at 30°C until emergence. All three incubations resulted in a highly significant delayed emergence of the French univoltine variety compared to the Canadian commercial variety. We conclude that the French univoltine trait is maintained under Saskatchewan conditions and could be a useful management tool to assist in the control of second generation.

18(b) TECHNICAL REPORT

(i) **Nature and scope of problem.**

The alfalfa leafcutting bee Megachile rotundata (Fab.) is widely used as a managed pollinator of alfalfa. This species originated in Eurasia and has been introduced into many countries (Parker 1982). The continued use of Megachile rotundata in different geographic areas may result in the natural selection of specifically adapted strains with altered diapause characteristics. To date there has been very little documentation of heritable differences between locally adapted strains although Hobbs and Richards (1976) state that natural selection in Lethbridge, Alta over an 8-year period reduced nondiapausing (second generation) bees of Megachile rotundata from 25 to 4.6% of cocoons.

Under conditions of field management, Megachile rotundata shows a variable emergence of the progeny of the spring generation. The portion of prepupae that do not enter diapause can exceed 40% when reared at Logan, UT (Parker 1979). These nondiapausing bees are referred to as a second generation and generally drift away from their shelters because of the sparseness of alfalfa bloom at the time of emergence (Tepedino and Parker 1986). Krunic (1972) found that cells from the earliest completed tunnels produced an increased percentage of second-generation bees.

Hobbs and Richards (1976) selected against bivoltinism over a 3-year period and produced strains with less than 1% second generation under field conditions at Lethbridge, Alta. They referred to the selected strain as univoltine in contrast to the unselected strain which produced approximately 5% second-generation bees. Leafcutting

bees imported from Canada produced up to 77% second-generation bees when reared in France (Tasei and Masure 1978, Tasei 1982). Univoltine strains of leafcutting bees native to France (Toe 1987) and Spain (Asensio 1982) produced virtually no second-generation bees under their field conditions. In a recent comparison of French univoltine and Canadian bees, Toe (1987) reported that the flight temperature of the French strains was 1.4° C less than the Canadian strain. Canadian bees were observed to fly later in the afternoon but were more restricted in their use of different plant species as pollen sources. The French strain also had different allelic frequencies for non-specific esterases and lactate dehydrogenase compared to the Canadian strain. When the Spanish leafcutting bee was introduced into Logan, UT it continued to show a univoltine phenotype (Parker 1982). Reciprocal crosses between Spanish and American strains identified a maternal effect for the univoltine trait (Parker and Tepedino 1982). Preliminary genetic analyses suggested that univoltinism was incompletely dominant and under polygenic inheritance (Parker and Tepedino 1982).

(ii) Objective

Our objective was to evaluate the heritability of the French univoltine trait under Saskatchewan field conditions.

(iii) Experimental design and analytical procedures

Bee strains. The univoltine strain was obtained from J.N. Tasei (INRA, Lusignan, France) in 1983. This strain originated as a wild trapped population from the region of Barbezieux in Charente (Toe 1987). One thousand cells were quarantined in Saskatoon, Sask., and reared in isolation from commercial bees. The absence of feral

populations in the Saskatoon environs precludes the use of field cages (Parker and Tepedino 1982) to maintain strain purity. By 1985, successive population increases at isolated field sites resulted in a sufficient population for diapause intensity evaluation. The commercial strain was obtained from a producer near Prince Albert, Sask. These bees originated from successive increases under commercial conditions over a 10-year period. It is assumed that any migration into this population (approx. 5,000,000 bees) from other commercial operations had a minimal effect on gene frequency because of the large size of the resident population.

Field sites. All field sites were within a 40 km radius of Saskatoon and locations were changed in each of the 3 years. In any given year, bee strains were placed greater than 2 km, but within 5 km of each other, in as identical locations as possible. In 1985 and 1986, plots were placed on dry land where alfalfa stands were managed as forage crops. In 1987, plots were placed at the edge of a pivot irrigation system used to irrigate a forage crop of alfalfa. In each year only one field site was used for any strain. One shelter was used for every 1.6 ha. Ten hives (30,000 tunnels) were used per shelter to accommodate approximately 20,000 bees/per ha. Under these conditions neither nesting sites nor pollen or nectar provisions limited population increase.

Bee management. Univoltine bee incubation was initiated 3 days earlier than commercial bee incubation so that emergence times were approximately synchronous. Cocoons harvested from the previous year were incubated at 30°C (60% RH) until approximately 20% of females

emerged. Then bees of both strains were placed in the field on the same day using identical 122 cm by 244 cm plywood shelters and 100 board polystyrene hives. Dates for placement in and removal from the field for both strains in years 1985, 1986 and 1987 were 25 June and 11 August, 30 June and 25 August, 21 June and August 22. After removal from the field hives were incubated (5°C above ambient in 1985 and at 25°C in 1986 and 1987) to allow complete spinning of cocoons prior to harvest. Hive incubation dates for 1985, 1986 and 1987 were 12 Aug. to 6, Sept., 25 Aug. to 13 Sept. and 23 Aug. to 13 Sept. respectively. Hives were then harvested and cells were either directly incubated at 30°C (fall incubation) or stored at 10°C to allow the maximum rate of diapause development (Krunic and Hinks 1972) for winter and spring incubation experiments.

Evaluation of diapause intensity. Field emergence of the univoltine strain cannot be effectively evaluated in Canada since our climatic conditions result in a low (approx. 5%) percentage of second-generation bees (Hobbs and Richards 1976; Krunic 1972). Thus we have evaluated the diapause intensity (Danks 1987) of French univoltine and Canadian commercial strains after different chilling times over a 3-year period. Diapause intensity has been defined (Danks 1987) as "the time required under given conditions before some measure of the end of diapause is observed (usually completion such as emergence or reproduction)". Adult emergence times from cells (cocoon containing prepupae) was used as a measure of diapause intensity. Cells of commercial and univoltine strains were incubated at 30°C and

60% RH and daily emergence time was determined for each of three seasonal periods: (i) fall incubation - initiated in September immediately after fall harvest of cells; (ii) winter incubation - initiated in December and (iii) spring incubation - initiated in April. Population sizes varied from 200 to 7 00 cells depending upon the year and time period being evaluated. Strain dependent emergence always resulted in unequal numbers of emergent bees. Thus all data was analyzed as an analysis of variance with unequal sample size.

(iv) **Results**

The 1986 emergence patterns for fall, winter and spring incubation are given in Figures 1, 2, and 3, respectively.

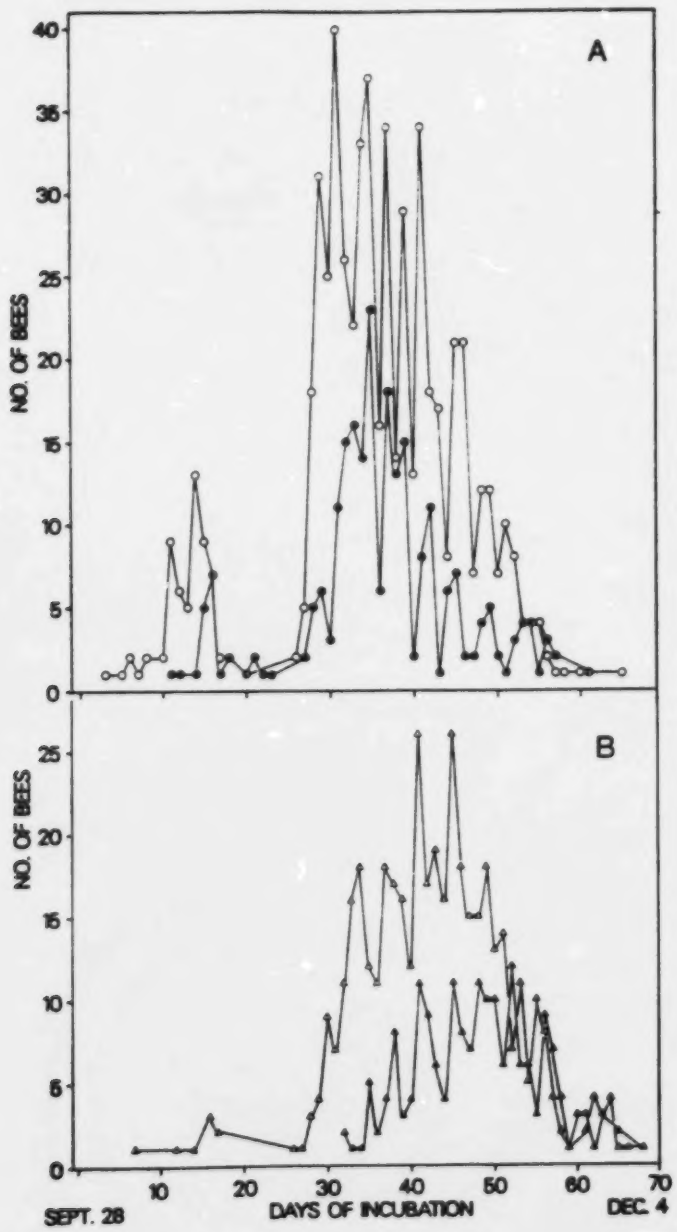

Fig. 1. Emergence pattern of 1986 fall incubation. A, Commercial strain - males (o), females (●). B, Univoltine strain - males (Δ), females (▲).

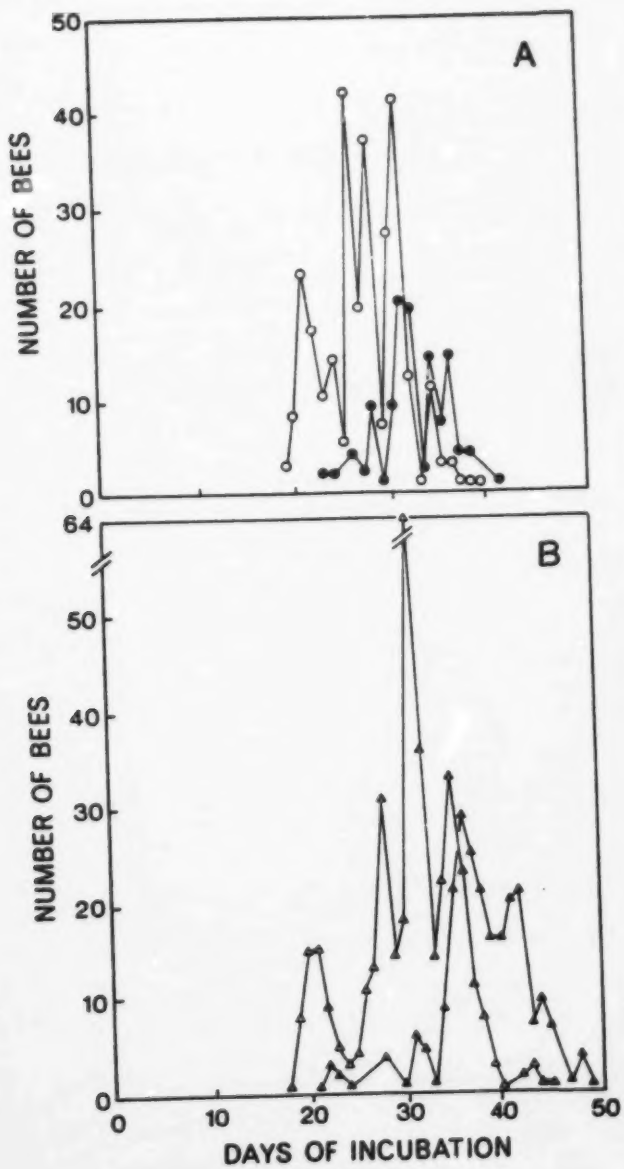

Fig. 2. Emergence pattern of 1986 winter incubation. A, Commercial strain - males (o), females (●). B, Univoltine strain - males (Δ), females (▲).

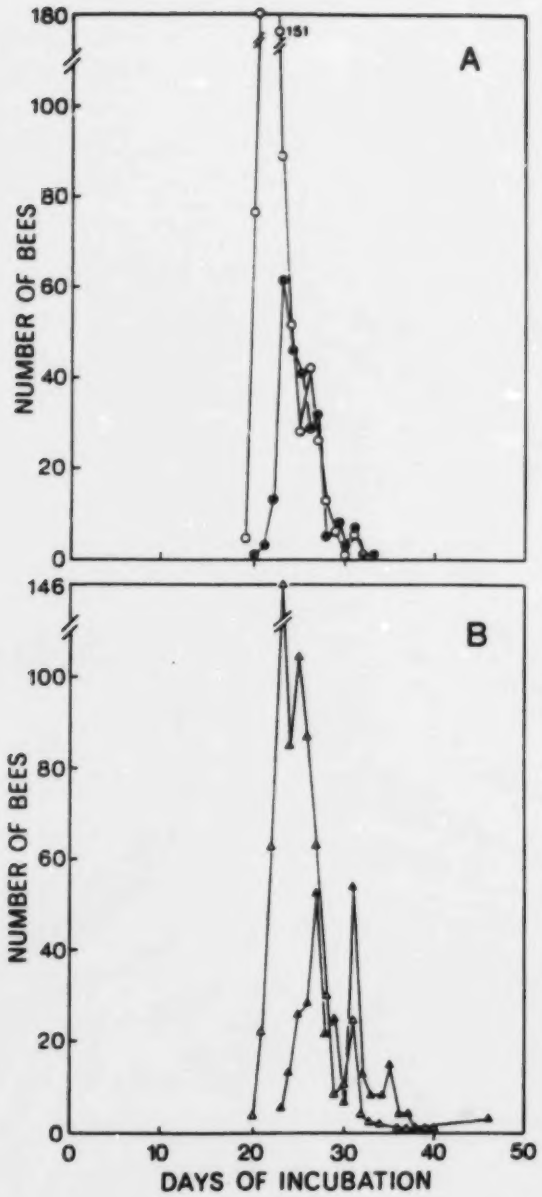

Fig. 3. Emergence pattern of 1986 spring incubation. A, Commercial strain - males (o), females (●). B, Univoltine strain - males (Δ), females (▲).

For both strains an increased chilling time resulted in a more synchronous and earlier emergence. The winter emergence pattern gave the best definition of the difference in diapause intensity of the two strains. A three-way analysis of variance of the 1985, 1986, and 1987 winter emergence pattern is given in Table 1.

Table 1. Analysis of variance of emergence times for the winter incubation experiments of 1985, 1986 and 1987.[1]

Strains	DF	F ratio	PR>F
Strain	1	3404	<0.0001
Sex	1	599	<0.0001
Year	2	76	0.0001
Strain x Sex	1	92	0.0001
Strain x Year	2	25	0.0001
Sex x Year	2	3	0.0327

[1] Total number of observations was 1807 univoltine and 1881 commercial bees.

All main and interactive components were significant. However, the main source of variability was attributed to differences between strains with a mean emergence date of 46.8 for the univoltine strain (1807 bees) and 36.6 for the commercial strain (1881 bees). The 1986-1987 mean emergence times for both sexes at each of the three incubation periods is given in Table 2.

Table 2. Average emergence times during the fall, winter and spring incubations of univoltine and commercial bees in 1985, 1986 and 1987.*

		Ave. Days to Emergence			
	Incubation	Univoltine		Commercial	
YEAR	Period	males	females	males	females
1986	Fall	42.8 (43.2)+	47.9 (47.9)	35.5 (38.1)	36.3 (38.4)
1987	"	45.1 (45.1)	54.0 (54.0)	35.3 (36.0)	40.5 (40.8)
1985	Winter	43.0	50.6	30.0	33.9
1986	"	45.4	52.3	36.3	38.6
1987	"	42.5	49.3	34.7	38.9
1985	Spring	23.8	26.9	21.3	22.9
1986	"	25.0	29.1	22.6	24.9
1987	"	28.9	33.8	24.0	27.5

* Treatment means within sexes of the two different strains were compared by a one-way analysis of variance. For each of the eight incubation periods the probability of a chance difference in mean emergence date between males and females of the two different strains was <0.0001.

+ Values enclosed by brackets are average days to emergence exclusive of any emergent bees prior to day 25.

Fall incubation. Prepupae for these experiments were not chilled at 10°C prior to incubation but were used immediately after harvest. Emergence occurred over a wide range, from 3 to approximately 70 days (Fig. 1). Incubation beyond 70 days resulted in sporadic and low emergence. We arbitrarily terminated incubation on day 67 in 1986, and on day 87 in 1987. The univoltine strain had an increased diapause intensity since univoltine male emergence was delayed by approximately 7 to 10 days and female emergence by 11 to 14 days compared with the commercial strain (Table 2). The difference in emergence within sexes between strains (e.g. 42.8 versus 35.5 days for 1986 males and 47.9 versus 36.3 days for 1986 females) was significant. In addition, an increased percentage of univoltine cells remained in diapause. Thus on day 67 (1986), 56.5% of univoltine cells had not emerged compared with 40.1% of commercial cells. Values for 1987 on day 87 were 32% of univoltine and 4.6% of commercial cells. Prepupae that undergo development are viable at 10°C for up to 19 days (Rank and Goerzen 1982). Since the development period is approximately 25 days, emergence prior to day 25 presumably results from prepupae that broke diapause prior to incubation at 30°C. Comparisons of diapause intensity under these conditions is still a valid and useful parameter as it is defined only on the basis of the average days to emergence. However, removal of bees that emerged prior to day 25 from the analysis still resulted in a significantly different emergence pattern between the two strains (Table 2).

Winter incubation. At the time of winter incubation, cells had undergone diapause development at 10°C for ca 12 weeks. In all 3

years the univoltine strain showed an increased diapause intensity compared with the commercial strain (Table 2). The difference in emergence time was greatest in 1985 where the increased univoltine average emergence times were 13 days for males and 17 days for females. In 1986 and 1987 values were 8 to 9 days for males and 10 to 14 days
for females.

Spring incubation. The prolonged cold storage (ca. 30 weeks) prior to spring incubation resulted in a much reduced emergence time (Table 2) and highly synchronous emergence (Fig. 3). Even after this long period of diapause development an increased diapause intensity of univoltine bees was maintained. Although the later emergence of the univoltine strain was only ca. 2 to 5 days for males and 4 to 6 days for females, these differences were highly significant.

Increased diapause intensity of univoltine females. The sex x strain interaction (Table 1) results from the late emergence pattern of univoltine females. The difference between univoltine male and female winter emergence for 1985, 1986 and 1987 was 7.6, 6.9 and 6.8 days whereas comparable values for the commercial strain were 3.9, 2.9 and 4.2 days (Table 2). Thus the average difference in emergence of sexes is approximately twice as large for the univoltine strain (average = 7.1 days) compared to the commercial strain (average = 3.5 days).

1988 fall and winter incubations

Comparisons between strains were made by plots of univoltine bees at Clavet and commercial bees at Warman. Fall and winter incubations confirmed the increased diapause intensity of the univoltine strain seen in 1986 and 1987.

(v) **Conclusions**

In view of the relative strain differences that were maintained under variable environmental conditions (Table 1, strain x year interaction) and developmental stages (Figs. 1, 2, 3, Table 2), we are confident that the trait of diapause intensity is heritable. Our results support the work of Parker (1982) and Parker and Tepedino (1982) on the heritability of voltinism in American and Spanish bees. Because of the requirement for field reproduction of this species, we have not been able to evaluate the underlying genetic basis for increased diapause intensity. Nevertheless, our results are qualitatively similar in that increased diapause intensity would be expected to result in a decreased second generation under field conditions.

Factors that have selected for different diapause intensities in bees from Barbezieux and those from Prince Albert are obscure. The most obvious difference in the two populations is that the French strain increased under natural conditions in a southern latitude (45.5° N) whereas the Saskatchewan population was managed artificially in a cooler, more northerly latitude (52° N). We presume that lack of suitable pollen and nectar sources exerted strong selection pressure against any emergent second generation in France. Under Saskatchewan management conditions, cells are often artificially removed from the field to prevent loss from second generation. This would have the effect of removing selection pressure for second generation and would be anticipated to select for the related trait of reduced diapause intensity that we have documented.

(vi) **Implications to Saskatchewan Agriculture**

The univoltine trait and the related characteristic of diapause intensity are clearly heritable under Saskatchewan environmental conditions. Use of the French univoltine strain in Saskatchewan would result in the virtual climination of an emergent second generation under field conditions. This should result in a small increase in bee yield (1.5%) and the prevention of damage to polystyrene hives. The 2 to 3 day later emergence during spring emergence of the French univoltine strain may provide a small additional time for control of the chalcid Pteromalic venustus. A lowering of chalcid re-paracitism during incubation by even 1% would be of considerable value to the producer.

An additional benefit that may result from the univoltine that accrues from the relationship between second generation and the spread of chalkbrood (Ascosphaera aggregata). It has been reported that emergent second generation bees chew through chalkbroad cadavers and thereby act as a vector in the spread of this disease (Vandenberg et al. 1980). The univoltine trait would act as an insurance against this mode of dissemination of chalkbroad.

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18(c) Non technical report for producers or extension personnel

I am not able to respond adequately to this request. As a result of our research I feel that I can tentatively recommend the use of the French univoltine strain for eventual use in Saskatchewan. However, our population sizes are not large enough for commercial distribution. Furthermore, the strain requires further selection and evaluation over a five-year period before a sound recommendation can be made.

18(d) Publications

1. Rank, G.H. and F.P. Rank. 1989. Diapause intensity in a French univoltine and a Saskatchewan commercial strain of Megachile rotundata. Can. Ent. 121: 141-148 (1989)

2. Rank, G.H. 1987. Diapause development of French univoltine bees. Proc. 7th Ann. Canadian Alfalfa Seed School. Saskatoon, Sask. pp. 53-54.

3. Rank G.H. and F.P. Rank. 1987. Reduced second generation of European univoltine leafcutter bees. 18th Ann. Interstate Seed School. Billings, Montana. 5 pp.